VIROLOGY 208, 789–794 (1995)

SHORT COMMUNICATION

Identification of Sequences in the Long Terminal Repeat of the Lymphoproliferative Disease Virus Required for Efficient Transcription

RONIT SARID,* ARNONA GAZIT,* STEVEN R. TRONICK,† and ABRAHAM YANIV*,[1]

*Department of Human Microbiology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv 69978, Israel; and †Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland 20892

Received November 14, 1994; accepted January 26, 1995

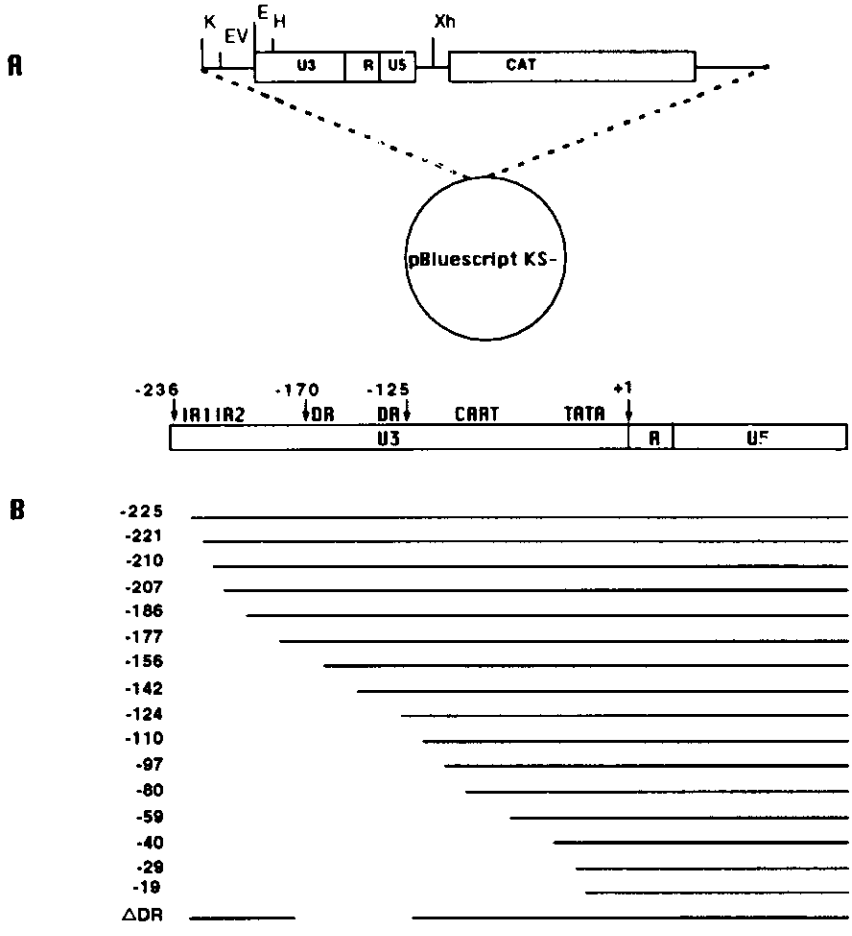
We analyzed the long terminal repeat (LTR) of the lymphoproliferative disease virus of turkeys for sequences that influence its promoter activity by using the chloramphenicol acetyltransferase assay. A series of LTR deletion mutants and recombinants between LTR and simian virus 40 regulatory sequences were used for these studies. Through transfection experiments, we identified a negative regulatory element residing at the 5′ end of the U3. The two imperfect direct repeats (DRs) located at nt −170 to −125 upstream of the RNA transcription site were identified as enhancer elements which could stimulate transcription of a heterologous promoter in an orientation independent manner. Specific interaction of nuclear factors with the DRs element was identified. The two DRs contain cArg motifs which are suggested to play a role in tissue specific expression of several cellular genes.

The lymphoproliferative disease (LPD) virus of turkeys (LPDV), is a type C-retrovirus which induces a naturally occurring disease in turkeys. The disease is characterized by rapidly developing lymphoproliferative lesions in the spleen, thymus, and pancreas, often resulting in 20% mortality in afflicted flocks (*1–4*). LPDV is a representative of a distinct class of avian retroviruses, which was shown to be evolutionarily related to the avian sarcoma-leukemia group of viruses (ASLV) (*5, 6*). LPDV is difficult to work with since a permissive cell culture for virus replication has not yet been found. However, virus derived from an infectious molecular clone of the integrated form of LPDV proviral DNA reproduced LPD *in vivo* (*7, 8*). This suggested that LPDV is a replication-competent virus, capable of inducing the disease in the absence of a defective transforming counterpart. Nucleotide sequence analysis (*6*) did not reveal the existence of a viral oncogene within the cloned LPDV genome, suggesting that LPD symptoms, which rapidly develop, are not induced by direct action of a viral oncoprotein. Replication-competent retroviruses can induce neoplastic diseases by insertional mutagenesis in which cellular protooncogenes are activated *in cis* by integrated retroviral long terminal repeats (LTRs) (*9, 10*). As part of our efforts to understand the molecular basis of LPDV pathogenesis, we undertook structure/function analysis of the LPDV LTR. Sequence analysis of the LTR predicted the presence of known eukaryotic transcriptional control elements such as an inverted CAAT box and a consensus TATA box promoter signal (*11*). Although sequences similar to the SV40 "core" enhancer motif (*12, 13*) were not found, imperfect direct repeats (DRs) were shown to be located at nt −170 to −154, and nt −140 to −125. Short direct repeats were often shown to be associated with enhancing activity in retroviral LTRs (for review see Ref. *14*).

To investigate the *cis*-acting regulatory elements within the LPDV LTR, the effect of sequential, progressive deletions of the U3 domain on the transcriptional activity of the viral LTR, was examined. Various 5′ deletion mutants were generated either by employing exonuclease III or by synthesizing various LTR domains using the polymerase chain reaction (PCR) (Fig. 1). Each mutant was assayed for the ability to direct the synthesis of chloramphenical acetyl transferase (CAT), following transfection of constructs into canine fetal thymus (Cf2Th) cells. The LPDV LTR was previously shown to be highly active in Cf2Th cells (*11*), similar to the level of transcription activity exhibited by the LTR of Rous sarcoma virus (RSV). The levels of CAT activity increased as a linear function of the amount of construct DNA used to transfect the cells up to 4 μg (~2 pmol) of DNA per 8 $\times 10^5$ cells (data not shown). pLTR(Δ-225)-CAT, pLTR(Δ-221)-CAT, and pLTR(Δ-210)-CAT, all of which had deletions in sequences upstream of position −210, expressed slightly higher CAT activity, as compared to that of pLPDV LTR-CAT which contained the entire LTR (Fig.

[1] To whom correspondence and reprint requests should be addressed. Fax: 972/3/642-2275.

0042-6822/95 $6.00


A
K
EV
E
H
Xh
U3
R
U5
CAT
pBluescript KS-
-236
-170
-125
+1
IR1 IR2
DR
DR
CART
TATA
U3
R
U5
B
-225
-221
-210
-207
-186
-177
-156
-142
-124
-110
-97
-80
-59
-40
-29
-19
ΔDR
C
RELATIVE CAT ACTIVITY
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
-225
-221
-210
-207
-186
-177
-156
-142
-124
-110
-97
-80
-59
-40
-29
-19
-124
ΔDR

1). Removal of an additional 3 bp [pLTR(Δ-207)-CAT] increased CAT activity up to approximately 1.5-fold, suggesting the presence of a negative regulatory element (NRE) within this region. Further deletions of additional 21 and 30 nt [pLTR(Δ-186)-CAT and pLTR(Δ-177)-CAT, respectively] slightly diminished transcription activity as compared to pLTR(Δ-207)-CAT, suggesting the presence of weak positive regulatory sequences in the region which spans nt −208 to −177. However, removal of additional 21 and 35 downstream nucleotides [pLTR(Δ-156)-CAT and pLTR(Δ-142)-CAT, respectively], which deleted most of the first DR sequences, resulted in a 50% reduction in CAT activity relative to that of the entire LTR, while further deletion [pLTR(Δ-124)-CAT] which removed also the second DR, reduced CAT activity by 90%. These results suggested that the region spanning nt −177 to −125, which contains the two DRs, includes strong control elements which positively regulate transcription from the viral promoter. To conclusively demonstrate that the loss of transcription activity was associated with the removal of the two DRs, a deletion mutant was constructed [pLTR(ΔDR)-CAT] in which the two DRs were removed by using the recombinant PCR method (*15*). The level of CAT activity was 20% of that of the entire LTR (Fig. 1). The fact that the level of CAT activity exhibited by pLTR(ΔDR)-CAT was slightly higher than that of pLTR(Δ-124)-CAT; may be due to the weak positive regulatory elements presumably located between nt −177 and nt −207. Removal of the CAAT box [pLTR(Δ-97)-CAT; pLTR(Δ-80-CAT] further reduced activity, while removal of TATA box [pLTR(Δ-19)-CAT] completely abolished it (Fig. 1). These results are consistent with those obtained with the RSV LTR (*16*), although deletions of promoter sequences in other retroviral LTRs, still resulted in residual transcriptional activity (*17–20*).

Experiments were further conducted to explore whether the two DRs function as a classical enhancer. Thus, their effect on the transcriptional activity of a heterologous promoter was investigated. Several chimeric constructs were prepared in which the DR sequences were inserted in both orientations upstream of the SV40 promoter, in an enhancerless CAT expression vector, pA10CAT (*21*). The inserted LPDV fragments, which included the first DR (DRI), the second DR (DRII), or both DRs (DRI + II), were generated by annealing complementary oligonucleotides (Fig. 2) in equimolar ratios. While placing DRI or DRII in the sense [pDRI(s)SV-CAT; pDRII(s)SV-CAT] or antisense [pDRI(as)SV-CAT; pDRII(as)SV-CAT] orientation, upstream of the SV40 promoter, augmented transcriptional activity up to 2- to 7-fold, the presence of both DRs in either orientation [pDRI + II(s)SV-CAT or pDRI + II(as)SV-CAT, respectively] exhibited additive effects and augmented SV40 promoter activity by up to 15-fold. These results suggested that the two DRs function as enhancer elements, similar to observations made with the RSV and Moloney murine leukemia virus (Mo-MuLV) LTRs in which enhancement of heterologous promoters by the viral DRs residing within the U3 of the LTR were observed (*16, 22*).

To determine whether the two DRs of the LPDV LTR function in factor recognition, electrophoretic mobility shift assays were performed. When a double strand (ds) oligonucleotide (nt −170 to −125) containing the two DRs (11) was labeled and incubated with nuclear extract from Cf2Th cells, five distinct slowly moving protein–DNA complexes were observed (Fig. 3, lane b). Three of them (designated by S) were specifically eliminated by addition of an unlabeled ds competitor (lanes c and d). In contrast, when the sense or the antisense single stranded (ss) oligonucleotides served as competitors, only the additional two complexes (referred to as NS) were eliminated. These data suggest that while the three S bands represent specific protein–DNA complexes, the two other NS bands represent nonspecific complexes formed in the presence of residual ss oligonucleotides present in the ds labeled probe. The three specific DNA–protein complexes detected might originate from several alternative modes of interaction: (a) three different proteins could bind to three independent sites within the DR domain; (b) one protein might bind to three distinct DNA sites; (c) one protein might bind to one specific site and several other proteins might multimerize with the DNA–

FIG. 1. Transcriptional activity of LPDV LTR mutants. (A) Schematic presentation of the pLPDV LTR-CAT. The entire LPDV LTR was inserted into the pBluescript KS(−) plasmid, upstream to the bacterial CAT gene and SV40 polyadenylation signal (21). E, *Eco*RI; EV, *Eco*RV; H, *Hin*dIII; K, *Kpn*I; X, *Xho*I. (B) Schematic presentation of LPDV LTR and the various LTR mutants. LTR mutants were generated as follows: pLPDV LTR-CAT was linearized by digestion with both *Eco*RV and *Kpn*I located upstream to the LTR and 5′ sequential deletions from the *Eco*RV site downstream were generated by using exonuclease III (Stratagene) (48). Additional deletion mutants were generated by PCR amplification using pLPDV-LTR CAT as a template, in the presence of an antisense primer which spans the 3′ end of the LTR and an appropriate sense primer designed to border the 5′ end of each deletion. The PCR products were inserted upstream to CAT gene, in a promoterless-CAT vector. pLTR(ΔDR)-CAT in which the two DRs were deleted, was constructed by the recombinant PCR technique (15). The integrity of all mutants was confirmed by dideoxynucleotide sequencing (49). The various sequential deletion mutants were designated according to their 5′ ends. (C) Transcriptional activity of LTR-CAT mutants. Cf2TH cells were transfected with 4 μg of each of the indicated plasmids, by a modification of the calcium phosphate–DNA coprecipitation technique, as previously described (11). At 48 hr after transfection, CAT activity was assessed according to Neumann *et al.* (50). The results were corrected for CAT activity exhibited by the promoterless CAT plasmid. The level of CAT activity of each construct was calculated as the quantity of acetylated chloramphenicol produced in 1-hr reaction during the logarithmic phase of the reaction, as previously described (51). The results were the mean of at least four independent transfections with two different preparations of plasmids, which were found to differ by not more than 10%. Relative CAT activity was calculated as the ratio between CAT activities obtained for each of the mutant plasmids, divided by the level of CAT activity exhibited by pLPDV LTR-CAT.

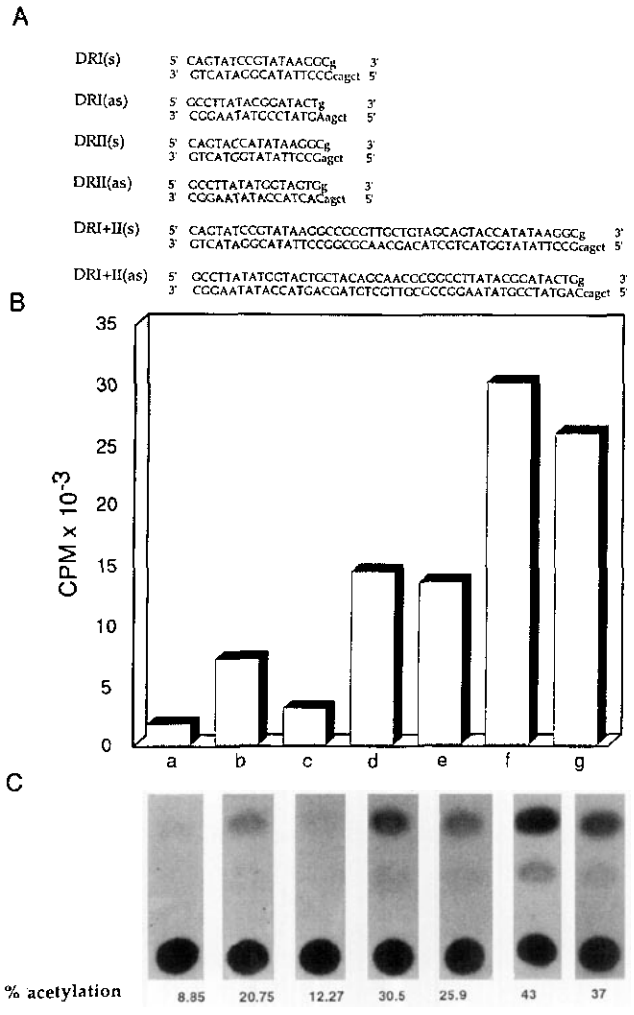


FIG. 2. Effect of the DRs elements of the LPDV LTR on the SV40 promoter-driven CAT gene expression in Cf2Th cells. (A) Complementary oligonucleotides which represent DRI, DRII, or both DRs modified at their 5′ ends to possess *Sall* cohesive ends after annealing were annealed and inserted in the sense [(pDRI(s)SV-CAT; pDRII(s)SV-CAT; pDRI + II(s)SV-CAT)] or antisense [(pDRI(as)SV-CAT; pDRII(as)SV-CAT; pDRI + II(as)SV-CAT)] orientation, upstream to SV40 promoter, in the enhancerless pA10CAT plasmid, described previously (28). Four micrograms of each plasmid were transfected into Cf2Th cells. (B) CAT levels were assessed according to Neumann *et al.* (50), as described in Fig. 1. (C) CAT levels were assessed employing ascending thin-layer chromatography (21) as described previously (52). Densitometric analysis was used to determine the levels of acetylated and nonacetylated compounds. Under the condition described, the assay was linear with respect to time and protein concentration, until about 70% conversion was achieved. (a) pA10CAT; (b) pDRI(s)SV-CAT; (c) pDRI(as)SV-CAT; (d) pDRII(s)SV-CAT; (e) pDRII(as)SV-CAT; (f) pDRI + II(s)SV-CAT; (g) pDRI + II(as)SV-CAT.

protein complex itself. Discrimination between the three possibilities should be possible after identification of the binding factors and localization of their specific sites of interaction within the 46-bp region, which spans the two DRs.

In the present study, we have used a transient expression assay to study the effects of sequential deletions of the U3 domain on the transcriptional activity of the LPDV LTR. Since no cell culture has been found to support LPDV replication, Cf2Th cells, which support efficient transcription from the LPDV LTR (*11*), were chosen for the transcription assays performed in the present study. Analysis of deletion mutants demonstrated that, whereas removal the 5′ 26 nt of the LPDV U3 region slightly increased transcriptional activity, further removal of sequences between nt −210 to −207 increased CAT activity up to 1.5-fold, thus suggesting this region to contain NRE. Although analysis of transcriptional regulatory elements in the U3 region of the RSV LTR has not revealed the presence of negative-acting sequences (*20, 23*), a NRE was detected within the U3 domains of reticuloendotheliosis virus, MoMuLV, human immunodeficiency virus, and equine infectious anemia virus (*24–28*). Sequence comparisons of this region with known NRE's did not reveal any similarity (data not shown). Although various cellular proteins were shown to bind to retroviral

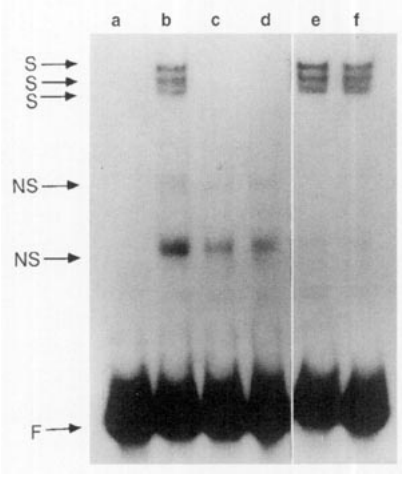


FIG. 3. Mobility shift assays using the 46-bp DRs element probe. The 46-bp ds oligonucleotide representing the two DRs (11), was generated by annealing complementary oligonucleotides in equal molar ratio, in a buffer containing 150 m*M* NaCl at a final ds DNA concentration of 10 μg/ml. The radioactive probe was prepared by 5′ end labeling with [γ-^{32}P]ATP (3,000 Ci/mmol; Amersham) and polynucleotide kinase. Nuclear extract was prepared from Cf2Th cells by the method according to Dignam *et al.* (53). Protein–DNA binding reaction mixture in a total volume of 20 μl contained the following: 0.1 ng of the end-labeled ds oligonucleotide probe (20,000 cpm), 12 m*M* Hepes (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) pH 7.9, 4 m*M* Tris–HCl, pH 7.9, 25 m*M* KCl, 1 m*M* EDTA, 1 m*M* dithiothreitol, 2 μl glycerol, and 2 μg poly(di)-poly(dC) (Pharmacia). Nuclear extracts (10 μg) either alone (lane b) or in the presence of 10 (lane c) or 1000 (lane d) molar ratios of unlabeled ds oligonucleotide competitor, or 10 molar ratios of ss sense (lane e) or antisense (lane f) oligonucleotide competitor, were added to the above described reaction mixtures. Following incubation at room temperature for 20 min, binding reactions were loaded on 6% nondenaturing polyacrylamide gel in a buffer containing 22 m*M* Tris–borate, 22 m*M* Boric acid, and 0.6 m*M* EDTA. Lane a contained the probe alone. Gels were dried and autoradiographed. S refers to DNA–protein complexes specifically eliminated by competition with the ds oligonucleotide; NS refers to nonspecific protein–DNA complexes, and F refers to free probe.

FIG. 4. Nucleotide sequence of the LPDV DRI and DRII compared to nt sequence of the RSV LTR enhancer (nt −110 to −89) (54), the 22-bp human c-*fos*, and Xenopous actin SREs (55). Dyad symmetry elements are underlined. The center of the dyad symmetry is marked by a star.

NREs (*29–32*), as well as to other cellular NREs (*33*), their nature and mode of activity were not established. However, it was speculated that NRE may stabilize binding of cell enhancer factor(s) to its DNA recognition site, thus, inhibiting subsequent interaction of the enhancer with downstream binding proteins.

Another important class of *cis*-acting sequences that activate transcription from the basal promoter are enhancers, which are generally positioned 100–200 bp upstream of the TATA box and specifically bind enhancing transcription factors. Although "core" enhancer elements were not found in the U3 domain of the LPDV LTR, deletion analyses could identify the presence of enhancing sequences within the DRs located in the U3 region. These DRs were shown to stimulate the activity of a heterologous promoter, in an orientation-independent fashion, thus, suggesting that they function as enhancer elements. Duplicated enhancer elements have been detected also within the U3 domain of RSV (*34*), shown to be evolutionarily related to LPDV (5). Although no extensive sequence relatedness was found between the LTR of LPDV and those of ASLV, sequence similarity was found in several regions such as the U3-R borders, the R region, the region containing the CAAT box, as well as the region which spans the two DRs (11). The enhancer located in the U3 of ASLV LTR was shown to be composed of four domains (for review see Ref. 14). One domain is similar to an SV40 core enhancer and another has homology to the octamer sequence found in the immunoglobulin gene enhancer. Several transcription factors, such as cellular enhancer factor 1 (EF-I) (*35, 36*), EF II (*37, 38*), EFIII (*39*), E2BP and E3BP (*40*), and F-I and F-III (*38*), have been shown to specifically bind to the distinct *cis*-acting enhancing elements of the ASLV U3. Upon closer inspection of the nucleotide sequence constituting the LPDV two DRs, we were intrigued by their strong sequence homology to the EFIII binding sites of the RSV LTR (Fig. 4), which include a CArg box [5′-CC (A/T)6 GG-3′] at nt −100 and a second CArg motif at nt −167 (*41, 42*). These binding sites were shown to exhibit both individual and combinatorial effects on basal activity of RSV LTR (*41*). A CArg motif is also present in the c-*fos* serum response element (SRE) (*43, 44*) and it was shown to bind a specific nuclear protein (serum response factor (SRF)) detected in both mammalian and amphibian cells. The EFIII binding site of the RSV LTR displays a strong sequence homology to SRE, and it was shown that the EFIII protein, which is presumably the avian homolog of SRF, binds to both the c-*fos* SRE and the EFIII binding site with equal affinity (*39*). This correlates with previous reports showing that the level of RSV transcripts in normal mammalian cells can be elevated by serum (*45*). Deletion studies performed in this study have demonstrated that the region of the two DRs, which constitutes two CArG motifs and thus presumably specifically binds the turkey homolog of SRF, is essential for full transcriptional activity of the LPDV LTR. Previous studies investigating the organotropism of LPDV suggested that LPDV expression is confined to lymphoid cells (*46*). Since it was shown that CArG motifs mediate different patterns of expression of their associated cellular genes *in vivo* (*42, 47*), it will be interesting to investigate the relevance of the two CArG motifs of the LPDV enhancer, in tissue-specific expression of LPDV in affected tissues.

ACKNOWLEDGMENTS

This work was supported by grants from the Fund for Basic Research, administered by the Israel Academy of Sciences and Humanities, the Israel Cancer Research Fund, the Israel Cancer Association, and the Eizen Foundation for Cancer Research.

REFERENCES

1. Biggs, P. M., McDougall, J. S., Frazier, J. A., and Milne, B. S., *Avian Pathol.* **7**, 131–139 (1978).
2. McDougall, J. S., Biggs, P. M., Shilleto, R. W., and Molne, B. S., *Avian Pathol.* **7**, 141–155 (1978).
3. Yaniv, A., Gazit, A., Ianconescu, M., Perk, K., Aizenberg, B., and Zimber, A., *J. Virol.* **30**, 351–357 (1979).
4. Gazit, A., Yaniv, A., Ianconescu, M., Perk, K., Aizenberg, B., and Zimber, A., *J. Virol.* **31**, 639–644 (1979).
5. Chajut, A., Sarid, R., Yaniv, A., Smythers, G. W., Tronick, S. R., and Gazit, A., *Gene* **122**, 349–354 (1992).
6. Sarid, R., Chajut, A., Gak, E., Kim, Y., Hixon, C. V., Oroszlan, S., Tronick, S. R., Gazit, A., and Yaniv, A., *Virology,* **204**, 680–691 (1994).
7. Gak, E., Yaniv, A., Chajut, A., Ianconescu, M., Tronick, S. R., and Gazit, A., *J. Virol.* **63**, 2877–2880 (1989).
8. Gak, E., Yaniv, A., Ianconescu, M., Tronick, S. R., and Gazit, A., *J. Virol. Methods* **28**, 147–154 (1990).
9. Ju, G., and Cullen, B. R., *Adv. Virus. Res.* **30**, 179–223 (1985).
10. Majors, J., *Curr. Top. Microbiol Immunol.* **157**, 49–85 (1990).
11. Gak, E., Yaniv, A., Sherman, L., Ianconescu, M., Tronick, S. R., and Gazit, A., *Gene* **99**, 157–162 (1991).
12. Laimins, L. A., Khoury, G., Gorman, C., Howard, B., and Gruss, P., *Proc. Natl. Acad. Sci. USA* **79**, 6453–6457 (1982).
13. Weiher, H., Konig, M., and Gruss, P., *Science* **219**, 626–631 (1983).
14. Luciw, P. A., and Leung, N. J., *In* "The Retroviridae" (J. A. Levy, Ed.), Vol. 1. pp. 159–298. Plenum Press, New York, 1992.
15. Higuchi, R., *In* "PCR Protocols — A Guide to Method and Applications" (M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, Eds.) Harcourt Brace Jovanovich Publishers (1990).
16. Laimins, L. A., Tsichlis, P., and Khoury, G., *Nucleic Acids Res.* **12**, 6427–6442 (1984).
17. Srinivasan, A., Reddy, E. P., Dunn, C. Y., and Aaronson, S. A., *Science* **223**, 286–289 (1984).

18. Gilmartin, G. M., and Parsons, J. T., *Mol. Cell. Biol.* **3,** 1834–1845 (1983).
19. Hanecak, R., Mittal, S., Davis, B. R., and Fan, H., *Mol. Cell. Biol.* **6,** 4634–4640 (1986).
20. Cullen, B. R., Raymond, K., and Ju, G., *Mol. Cell. Biol.* **5,** 437–447 (1985).
21. Gorman, C. M., Moffat, L. F., and Howard, B. H., *Mol. Cell. Biol.* **2,** 1044–1051 (1982).
22. Laimins, L. A., Gruss, P., Pozzatti, R., and Khoury, G., *J. Virol.* **49,** 183–189 (1984).
23. Luciw, P. A., Bishop, J. M., Varmus, H. E., and Capecchi, M., *Cell* **33,** 705–716 (1983).
24. Rosen, C. A., Sodroski, J. G., and Haseltine, W. A., *Cell* **41,** 813–823 (1985).
25. Hirano, A., and Wong, T., *Mol. Cell. Biol.* **8,** 5232–5244 (1988).
26. Flanagan, J. R., Kreig, A. M., Max, E. E., and Khan, A. S., *Mol. Cell Biol.* **8,** 739–746 (1989).
27. Flanagan, J. R., Becvker, K. G., Ennist, D. L., Gleason, S. L., Driggers, P. H., Levi, B. Z., Appella, E., and Ozato, K., *Mol. Cell. Biol.* **12,** 38–44 (1992).
28. Sherman, L., Yaniv, A., Lichtman-Pleban, H., Tronick, S. R., and Gazit, A., *J. Virol.* **63,** 4925–4931 (1989).
29. Calvert, I., Peng, Z., Kung, H., and Raziuddin., *Gene* **101,** 171–176 (1991).
30. Yamamoto, K., Mori, S., Okamoto, T., Shimotohno, K., and Kyogoku, Y., *Nucleic Acids Res.* **22,** 6107–6112 (1991).
31. West, M., Mikovits, J., Princler, G., Liu, Y., Ruscetti, F. W., Kung, H., and Raziuddin., *J. Biol. Chem.* **267,** 24948–24952 (1992).
32. Giacca, M., Gutierrez, M. I., Menzo, S., Fagagna, F. D., and Falaschi, A., *Virology* **186,** 133–147 (1992).
33. Miyazaki, J., Appella, E., and Ozato, K., *Proc. Natl. Acad. Sci. USA* **83,** 9537–9541 (1986).
34. Gowda, S., Rao, A. S., Kim, Y. W., and Guntaka, R. V., *Virology* **162,** 243–247 (1988).
35. Ryden, T. A., and Beemon, K., *Mol. Cell. Biol.* **9,** 1155–1164 (1989).
36. Faber, M., and Sealy, L., *J. Biol. Chem.* **265,** 22243–22254 (1990).
37. Sealy, L., and Chalkley, R., *Mol. Cell. Biol.* **7,** 787–798 (1987).
38. Goodwin, G. H., *J. Virol.* **62,** 2186–2190 (1988).
39. Boulden, A., and Sealy, L., *Virology* **174,** 204–216 (1990).
40. Kenny, S., and Guntaka, R. V., *Virology* **176,** 483–493 (1990).
41. Boulden, A. M., and Sealy, L. J., *Mol. Cell. Biol.* **12,** 4769–4783 (1992).
42. Zachow, K. R., and Conklin, K., *J. Virol.* **66,** 1959–1970 (1992).
43. Triesman, R., *Cell* **42,** 889–902 (1985).
44. Mohun, T. J., Garrett, N., and Triesman, R. H., *EMBO J.* **10,** 933–940 (1987).
45. Dutta, A., Stoeckle, M. Y., and Hanafusa, H., *Genes Dev.* **4,** 243–254 (1990).
46. Gazit, A., Schwarzbard, Z., Yaniv, A., Ianconescu, M., Perk, K., and Zimber, A., *Int. J. Cancer* **29,** 599–604 (1982).
47. Taylor, M., Triesman, R., Garrett, N., and Mohun, T., *Development* **106,** 67–78 (1988).
48. Hemikoff, S., *Methods Enzymol.* **155,** 9–18 (1987).
49. Sanger, F., Nicklen, S., and Coulson, A. R., *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467 (1977).
50. Neumann, J. R., Morency, C. A., and Russian, K. O., *BioTechniques* **5,** 444–447 (1987).
51. Noiman, S., Gazit, A., Tori, O., Sherman, L., Miki, T., Tronick, S. R., and Yaniv, A., *Virology* **176,** 280–288 (1990).
52. Sherman, L., Gazit, A., Yaniv, A., Kawakami, T., Dahlberg, J. E., and Tronick, S. R., *J. Virol.* **62,** 120–126 (1988).
53. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G., *Nucleic Acids Res.* **11,** 1475–1489 (1983).
54. Swanstrom, R., DeLorbe, W. J., Bishop, J. M., and Varmus, H. E., *Proc. Natl. Acad. Sci. USA* **78,** 124–128 (1981).
55. Triesman, R., *EMBO J.* **6,** 2711–2717 (1987).